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Pharmaceutical properties of insulin conjugate with glucuronylglucosyl-β-cyclodextrin

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In the clinical field, insulin has been used for treatment of diabetes. However, insulin often shows low physicochemical stability, adsorption onto tubes and enzymatic degradation in injection site or blood. Previously, we demonstrated that cyclodextrins (CyDs), especially branched β-CyDs, improve the pharmaceutical properties of insulin through complexation with its aromatic residues. However, little has been reported on the insulin conjugate with branched β-CyD. In the present study, to improve the pharmaceutical properties of insulin, we newly prepared 6-*O*-α-(4-*O*-α-D-glucuronyl)-D-glucosyl-β-CyD (GUG-β-CyD) conjugate with insulin, and evaluated its thermal or enzymatic stability and adsorption onto glass or polypropylene tube [1]. To prepare GUG-β-CyD-insulin conjugate, insulin and activated GUG-β-CyD were reacted in DMF/water (pH 10) for 10 min at room temperature (Fig. 1A). Preparation of GUG-β-CyD-insulin conjugate was confirmed by a MALDI-TOF mass spectrum. An inclusion ability of CyD in GUG-β-CyD-insulin conjugate was examined by measuring fluorescence spectrum in the presence of 2-*p*-toluidinylnaphthalene-6-sulfonate(TNS). The conformation of insulin in GUG-β-CyD-insulin conjugate was confirmed by a circular dichroism (CD) spectrum. In the adsorption study, GUG-β-CyD-insulin conjugate solution was placed in a glass or a polypropylene tube. After standing the sample for 2 h at 25 °C, the absorbance of the solution was

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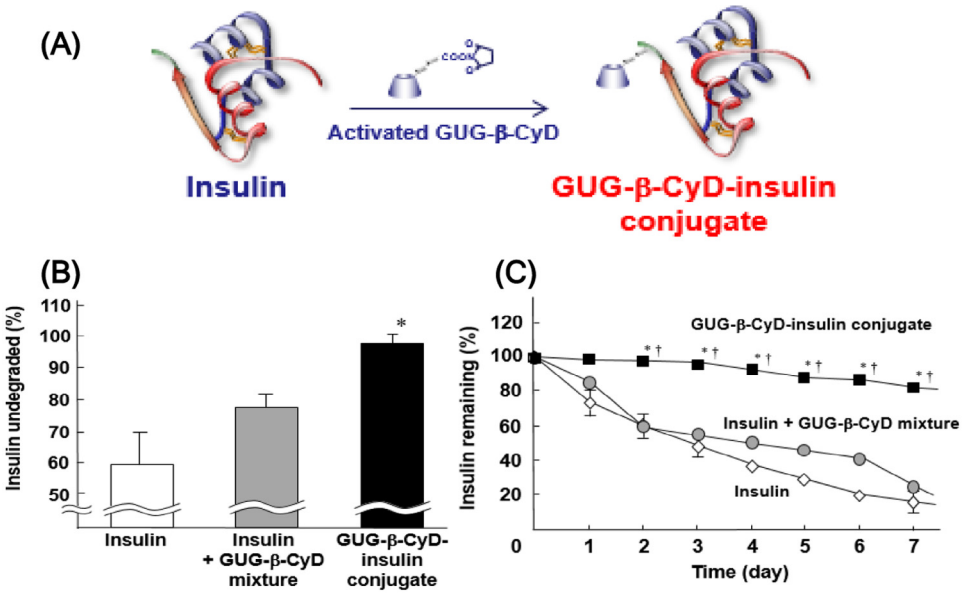

Fig. 1 – (A) Preparation pathway of GUG-β-CyD-insulin conjugate and its (B) enzymatic and (C) thermal stability. Each value represents the mean ± S.E.M. of 3–6 experiments. *$P < 0.05$ *versus* insulin. †$P < 0.05$ *versus* insulin + GUG-β-CyD mixture.

measured by a spectrophotometer (280 nm). The thermal or enzymatic stability of GUG-β-CyD-insulin conjugate was evaluated after heating at 50 °C or incubation with trypsin at 37 °C, and then the intact insulin level was measured by a spectrophotometer or HPLC.

A new peak derived from GUG-β-CyD-insulin conjugate was observed in a MALDI-TOF mass spectrum. The fluorescence intensity of TNS markedly increased in the presence of GUG-β-CyD-insulin conjugate, suggesting that CyD in GUG-β-CyD-insulin conjugate possesses inclusion ability. Also, according to the result of a CD spectrum, conformation of insulin in the conjugate was retained. GUG-β-CyD-insulin conjugate adsorbed onto a glass and a polypropylene tube only very slightly. Thermal and enzymatic stability of GUG-β-CyD-insulin conjugate was markedly improved, compared to that of insulin alone or the mixture (Fig. 1B,C).

Consequently, these results suggest that the conjugation with GUG-β-CyD could be useful for improvement of some pharmaceutical properties of insulin.

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